BIOSYNTHESIS OF THE PYRIDINE RING OF RICININE FROM SUCCINATE AND OTHER LABELED COMPOUNDS

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Leete and Leitz (1957) have shown that nicotinic acid-7-C^{14} gives rise to ricinine (N^1-methyl-3-cyano-4-methoxy-2-pyridone), the alkaloid produced by the castor plant Ricinus communis L. The carbon-14 was located in the cyano group. Recently Waller and Henderson (1961) showed that the pyridine nucleus of ring labeled nicotinic acid and nicotinamide can become the pyridine ring of ricinine. Based on these results ricinine synthesis appears to be a good system to use for the study of the formation of the pyridine nucleus in higher plants. It is well known that nicotinic acid or nicotinamide may arise from the indole nucleus of tryptophan in animals (Krehl, et al., 1949) and Neurospora (Beadle, et al., 1947). However, this biological transformation does not occur in higher plants (Grimshaw and Marion, 1958) or in certain bacteria (Yanofsky, 1954).

Experimental Procedure:

To study the biological transformation of suspected labeled precursors into ricinine these compounds were injected into one of the hollow internodular spaces of young castor plants. A hypodermic needle was inserted at the top of the internode of a castor plant to serve as a vent. An aqueous solution of the labeled compound (25-50 µl.) was injected at the bottom of the internode using a micro syringe. The solution was completely absorbed within a few minutes after injection (the injected compounds had no effect on alkaloid production; Table I, footnote).

Castor plants of the Cimarron variety were grown on a Port clay loam

soil under irrigation during the summer of 1960. The ricinine was isolated from the plants 24 to 96 hours after the compounds had been injected and its specific activity determined as reported (Waller and Henderson, 1961).

Results and Discussion:

The data shown in Table I when compared to the results obtained from nutrient solution cultures (Waller and Henderson, 1961) indicates that the incorporation of radioactivity from an isotopically labeled precursor was higher when the compound was injected. Nicotinic acid-7-C^{14} appears to be on the main biosynthetic pathway based on its high incorporation into ricinine (11.0% and 27.5% after 24 and 96 hours, respectively). This high extent of incorporation may be considered all the more significant since in the biosynthetic pathway from nicotinic acid to ricinine an extensive series of reactions are involved.

Since nicotinic acid is a precursor of ricinine it was of interest to extend these studies to include the biogenesis of the pyridine ring by studying the incorporation of small isotopically labeled molecules as succinate, propionate, acetate, β-alanine and glycerol. To measure the relative importance of these compounds in a given experiment the per cent of incorporation of nicotinic acid-7-C^{14} into ricinine was given a value of 1000; the per cent of incorporation of radioactivity from other compounds was compared to it to give the "Nicotinic Acid Index."

Succinate-2,3-C^{14} was the most efficient precursor of ricinine (Table II). Succinate-1,4-C^{14} was incorporated to a much smaller extent. Because of the position of the label of succinate-1,4-C^{14} this would be expected after the compound has made several passes through the tricarboxylic acid cycle. All of the carbon-14 from succinate-2,3-C^{14} was found to be in the pyridone ring of ricinine. For example, in a typical experiment, ricinine, biosynthesized from succinate-2,3-C^{14} having a specific activity of 20.65 mμc/mmole was treated with 57.4% H_2SO_4 to remove the cyano group (Winterstein, et al., 1917) the resulting N-methyl-4-methoxy-2-pyridone had a specific activity of 20.4

Table I

Preliminary Experiments to Ascertain if Known Precursors of Ricinine Could Be Incorporated into the Alkaloid When Injected into the Plant*

Compound Injected	Duration of Experiment (Hrs.)	Precursor		Ricinine			
		Specific Activity mc/mmole	μ moles	Crude Yield μ moles	Specific Activity mμc/mmole	% Incorporation	Nicotinic Acid Index
Glycine-2-C^{14}	24	1.68	0.60	268	18.5	0.45	41
Glycine-2-C^{14}	96	1.68	0.60	275	17.5	0.48	17
DL-Glutamic Acid-2-C^{14}	24	0.54	1.85	212	27.5	0.59	54
Nicotinic Acid-7-C^{14}	24	5.88	0.11	283	257.5	11.0	1,000
Nicotinic Acid-7-C^{14}	96	5.88	0.11	300	611.7	27.5	1,000
Distilled Water Control	48	----	1.11	209	---	---	---
Distilled Water Control	120	----	1.11	379	---	---	---

* Compounds were injected into the second internode of 22 day-old castor plants on 7/1/60. The plants were at the fifth nodal stage of development and some of them had retained their cotyledonary leaves. The quantity of ricinine isolated was 0.079 ± 0.006% on a fresh weight basis.

TABLE II

INCORPORATION OF C^{14} FROM LABELED COMPOUNDS INTO RICININE

Precursor			Ricinine			
Compound Injected	Specific Activity mc/mmole	μ moles	Crude Yield μ moles	Specific Activity mμc/mmole	% Incorporation	Nicotinic Acid Index
Succinate-2,3-C^{14}	13.7	0.62	237	1,951.6	5.44	198
Succinate-1,4-C^{14}	5.0	0.80	269	153.5	1.03	33
Na Propionate-1-C^{14}	2.9	1.38	142	37.2	0.13	4
Na Propionate-2-C^{14}	2.21	1.81	206	477.2	2.46	78
Na Propionate-3-C^{14}	0.75	4.60	133	242.7	0.94	30
Na Acetate-1-C^{14}	1.9	3.65	45	337.5	0.22	7
Na Acetate-2-C^{14}	1.9	3.65	83	498.0	0.66	21
Glycerol-1,3-C^{14}	3.8	0.79	376	62.0	0.78	25
Glycerol-2-C^{14}	4.3	0.70	107	35.4	0.13	4
β-alanine-1-C^{14}	1.00	9.50	82	249.3	0.22	7
β-alanine-2-C^{14}	1.00	2.07	127	208.3	1.27	40

Compounds were injected into the second internode of castor plants at the fifth to seventh nodal stage of development. The duration of each experiment was 96 hours. The yield of ricinine was dependent upon uncontrollable agronomic conditions but in each experiment control plants gave similar yields.

mμc/mmole. The O- and the N-methyl groups were removed in succession according to the micro-methods of Pregl and the specific activity was determined on the tetramethylammonium iodide. An insignificant amount (less than 2%) of radioactivity was found in the methyl carbons. The activity from ricinine biosynthesized from succinate-1,4-C^{14} was distributed as follows: 25% in the cyano group, none in either the O- or the N-methyl groups and 75% in the pyridone ring of N-methyl-4-methoxy-2-pyridone. These findings closely parallel those of Ortega and Brown (1960) in the biosynthesis of nicotinic acid from E. coli. The distribution of the label in ricinine formed from succinate was the same in flowering and non-flowering plants.

The results from the incorporation of propionic acid into ricinine suggest that this compound may give rise to succinic acid via a β-oxidative pathway (Giovanelli and Stumpf, 1957). The amount of radioactivity incor-

porated into ricinine from propionate depended upon the position of the label. The order observed in several different experiments with non-flowering plants was Na-propionate-2-C^{14} > Na-propionate-3-C^{14} > Na-propionate-1-C^{14} [1]. In experiments with flowering plants the per cent of incorporation of Na-propionate-1-C^{14}, -2-C^{14} or -3-C^{14} was essentially the same (0.38%, 0.37% and 0.21% respectively). This difference between flowering and non-flowering plants is striking.

The higher incorporation of radioactivity from acetate-2-C^{14} compared to that of acetate-1-C^{14} is consistent with acetate conversion to succinate through the tricarboxylic acid cycle.

β-alanine-2-C^{14} was incorporated more than β-alanine-1-C^{14}. If β-alanine proceeds through propionate then the 2-labeled compound would be incorporated to a greater extent.

The incorporation of glycerol, although low, supports and extends the findings on incorporation of glycerol-1,3-C^{14} into the pyridine ring of nicotinic acid produced by E. coli (Ortega and Brown, 1960) and into the pyridine ring of nicotine (Griffith, et al., 1960). The difference in the per cent of incorporation of radioactivity from glycerol-1,3-C^{14} and glycerol-2-C^{14} may be explained by its conversion to acetate through glycolysis. Glycerol-1,3-C^{14} and glycerol-2-C^{14} are not incorporated into ricinine to any significant extent (less than 0.02%) in flowering plants.

SUMMARY

The biosynthesis of the pyridine ring of the alkaloid ricinine has been studied by injecting an aqueous solution of the test compound into the hollow internodular space of a young castor plant. By this technique 11.0% and 27.5% of nicotinic acid-7-C^{14} was incorporated into ricinine after 24 and 96 hours, respectively. Studies on the incorporation of succinate, propionate,

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[1] Similar results have been reported by Anwar, R. A., Griffith, T. and Byerrum, R. U., Fed. Proc., 20, 374 (1961).

acetate, glycerol and β-alanine showed the following order of efficiency as precursors of ricinine: succinate, propionate, β-alanine. Acetate and glycerol were incorporated approximately to the same extent. All of the radioactivity in ricinine biosynthesized from succinate-2-3-C^{14} was found to reside in the pyridone ring. The label in the alkaloid formed from succinate-1,4-C^{14} was located 75% in the pyridone ring and 25% in the cyano group.

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